NMDA receptor- and metabotropic glutamate receptor-dependent synaptic plasticity induced by high frequency stimulation in the rat dentate gyrus *in vitro*

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1. The mechanisms of long-term potentiation (LTP) and long-term depression (LTD) induced by brief high frequency stimulation (HFS), paired with a particular pattern and amplitude of depolarisation has been investigated in the medial perforant pathway of the dentate gyrus of the 2- to 3-week-old rat hippocampus *in vitro*.

2. *N*-Methyl-D-aspartate (NMDA) receptor (NMDAR) activation was measured quantitatively during HFS-induced NMDAR-dependent LTP, LTD and at the LTD–LTP crossover point in order to test the hypothesis that the induction of the particular form of plasticity depends on the intensity of NMDAR activation.

3. The induction of LTD, the LTD–LTP crossover point and LTP was associated with an increasing NMDAR charge transfer.

4. In addition to the NMDAR-dependent LTD, a group I metabotropic glutamate receptor (mGluR)-dependent LTD could be induced by high intensity HFS paired with depolarisation under conditions of NMDAR inhibition.

5. The induction of mGluR-dependent LTD requires membrane depolarisation, Ca^{2+} influx via L-type Ca^{2+} channels and a rise in intracellular Ca^{2+}.

6. Quantal analysis involving minimal stimulation demonstrated that the mGluR-dependent LTD induction was associated with a decrease in potency and an increase in failure rate.

LTP and LTD are long-lasting forms of synaptic plasticity involving an increase or decrease, respectively, in synaptic transmission. Both LTP and LTD have forms dependent upon the activation of NMDARs (Collingridge *et al.* 1983; Dudek & Bear, 1992*a*,*b*; Mulkey & Malenka, 1992). In addition, although initially controversial (Selig *et al.* 1995), there is now an abundance of evidence that certain forms of LTD induction are dependent upon the activation of mGluRs independently of NMDAR activation (Bolshakov & Siegelbaum, 1994, 1997; O'Mara *et al.* 1995; Oliet *et al.* 1997; Wang *et al.* 1997).

LTD is most commonly induced by prolonged (several minutes) low frequency stimulation (LFS) at 1–5 Hz, and LTP by brief high frequency stimulation (HFS) at 50–200 Hz (Bliss & Lomo, 1973; Bliss & Collingridge, 1993). The induction of LTD and LTP by such differing frequencies has been quantified formally in a frequency–response function (Bear, 1995), describing the smooth transition from LTD to LTP which occurs as the frequency of stimulation is increased from 1 to 200 Hz, with an LTD–LTP crossover point at 10–20 Hz. It has been hypothesised that the transition from LTD to LTP with increasing frequency of stimulation is associated with a higher level of activation of NMDARs and of associated Ca^{2+} influx (Bear & Malenka, 1994; Bear, 1995). Qualitative support for this theory was provided by the study of Cummings *et al.* (1996), in which LTP induced by brief HFS under control conditions was converted to LTD induction in the presence of partial NMDAR blockade by D-2-amino-phosphonopentanoate (AP5). However, quantitative measurements of NMDAR activation during the induction of plasticity have not so far been carried out to verify this theory. NMDAR activation is particularly difficult to measure during the induction of LTD by LFS because the NMDAR component of single excitatory postsynaptic currents (EPSCs) evoked at 1 Hz is very small. In the present study, we have devised a stimulation protocol for the induction of NMDAR-dependent LTD, LTP and the LTD–LTP crossover point in which a brief constant amplitude HFS is paired with postsynaptic depolarisation, the particular induction of LTP, LTD or the LTD–LTP crossover point being determined by changes in the pattern or amplitude of postsynaptic depolarisation. The presynaptic stimulation strength during the HFS, and therefore the number of synapses stimulated, was kept at a constant level. The

summation of NMDAR-mediated currents which occurred during the HFS enabled quantitative measurements of NMDAR activation to be made and correlated with the amplitude and direction of synaptic plasticity. In addition, we have characterised an NMDAR-independent LTD which was evoked by HFS under conditions of blockade of NMDAR.

METHODS

All experiments were carried out on transverse slices of rat hippocampus (Wistar rats, aged 2–3 weeks, weight 40–80 g). Animal use was approved by the Bioresources Committee, Trinity College, Ireland. Rats were killed by decapitation and the brains rapidly removed and placed in cold oxygenated medium (95% O_2–5% CO_2). Slices were cut at a thickness of 350 μm using a Campden vibroslice and placed in a holding container containing oxygenated medium at room temperature (20–22 °C). The slices were then transferred as required into a submerged recording chamber and continuously superfused at a rate of 8 ml min^{-1} at 32 °C.

The control medium contained (mM): NaCl, 120; KCl, 2.5; NaH_2PO_4, 1.25; $NaHCO_3$, 26; $MgSO_4$, 2.0; $CaCl_2$, 2.0, and D-glucose, 10. All solutions contained 100 μM picrotoxin (Sigma) to block $GABA_A$ receptor-mediated activity. AP5 was obtained from Tocris Cookson. The patch-clamp electrode, resistance 5–8 MΩ, contained (mM): potassium gluconate, 130; KCl, 10; EGTA, 10; $CaCl_2$, 1; $MgCl_2$, 3; Hepes, 20; Mg-ATP, 5; Na-GTP, 0.5; QX 314, 5; and the pH was adjusted to 7.2 (using KOH). The mGluR antagonists (*R,S*)-α-methyl-4-carboxyphenylglycine (MCPG), (*R,S*)-1-aminoindan-1,5-dicarboxylic acid (AIDA) and (2*S*)-α-ethylglutamic acid (EGLU) were obtained from Tocris Cookson.

Whole-cell recordings from dentate granule cells were made using an Axopatch-1D amplifier (3 kHz low pass Bessel filter), as described previously (O'Connor *et al.* 1995). Series resistance (R_s) varied from 15 to 25 MΩ, as measured directly from the amplifier or as measured directly in several cells from the peak amplitude of the resistive current I_c (without low pass filtering), as $R_s = ER/I_c$, where ER is the amplitude of the test pulse, usually 10 mV. The mean input resistance was 286 ± 36 MΩ, $n = 53$, and the mean resting potential −70 ± 4 mV, $n = 57$. The input resistance was monitored continuously, and the recording terminated if it varied by more than 10%. Test EPSCs were recorded at a holding potential of −70 mV in response to stimulation of the medial perforant pathway at a control frequency of 0.05 Hz, with the stimulation intensity adjusted to evoke an EPSC which was about 30% of the maximum amplitude, usually about 100 pA. Full experiments were carried out providing that certain criteria were met. These included a resting membrane potential of at least −65 mV, a high input resistance (at least 200 MΩ) and a low threshold and steep input–output curve for the EPSCs. Recordings were analysed using pCLAMP (Axon Instruments). Values are the means ± S.E.M., and Student's t test was used for statistical comparisons.

The presynaptic level of stimulation during baseline recordings was set to evoke test EPSCs of ~100 pA. Synaptic plasticity was evoked by HFS stimulation protocols, consisting of the pairing of a series of five trains of HFS (each of eight stimuli at 200 Hz, inter-train interval 200 ms) with step depolarisations from a holding potential of −70 mV. The presynaptic level of stimulation during HFS was increased threefold from the test level, sufficient to evoke EPSCs of ~300 pA, and was set at the same intensity for the induction of LTP, LTD and the LTD–LTP crossover point. The holding potential was always −70 mV, and the imposed postsynaptic depolarisation was one of three types. Firstly, for the induction of LTP, a single step of 1.1 s to −30 mV was applied during the HFS trains (with no repolarisation after each train). Secondly, for the induction of the LTD–LTP crossover point, the stimulus was a series of five steps to −30 mV, with each step depolarisation of 40 ms duration applied at 5 Hz and the depolarisation phase coinciding with each train of HFS. Thirdly, for the induction of LTD, a single step of 1.1 s to −50 mV was applied during the HFS trains. All values of plasticity were measured at 25 min post-HFS. In order to avoid any long-term changes of NMDAR activity by HFS, the summated NMDAR component was measured in one of two ways. Either HFS was initially given in the presence of AP5 to block long-term changes, the AMPA receptor (AMPAR) component measured, and then subtracted from the total AMPAR and NMDAR component obtained from the second HFS given after washout of AP5, or the NMDAR component was measured in cells from different slices of the same animals, using identical conditions to experiments in which LTP was induced. The two methods gave very similar values of the NMDAR component.

In minimal stimulation experiments, the frequency of test stimulation was set at a higher level of 0.1–0.2 Hz in order to ensure an adequate number of EPSCs for analysis. The initial stimulus intensity was set at a level below which EPSCs were evoked. The intensity was then increased very slowly until the lowest level that evoked EPSCs and failures was detected. Minimal stimulation was only accepted if at least 10% of trials resulted in failures.

RESULTS

Induction of LTP by a HFS–depolarisation pairing protocol evoking large NMDAR activation

A pairing of HFS and postsynaptic depolarisation was an effective stimulus for the induction of NMDAR-dependent LTP providing that large NMDAR activation was evoked by the pairing procedure. This was achieved by pairing a series of HFS trains with a single strong step depolarisation from −70 to −30 mV and duration 1.1 s (see Methods for details). LTP was induced by this protocol with a mean amplitude of 165 ± 19.1% ($n = 7$, $P < 0.05$; Fig. 1*A*). The LTP was NMDAR dependent, being blocked by AP5. Thus, the pairing of HFS and depolarisation in the presence of the NMDAR antagonist AP5 (100 μM) did not induce LTP, but rather an NMDAR-independent LTD measuring 37.9 ± 15.6%, $P < 0.05$ ($n = 6$; Fig. 1*B*). The properties of this LTD are investigated below.

During each HFS in control media, successive EPSCs summated temporally to form a prolonged current waveform, composed of both AMPAR- and NMDAR-mediated components (Fig. 1*C*i) (the amplitude of individual EPSCs declined rapidly during each train due to strong presynaptic short-term depression at this synapse; McNaughton, 1980). Similar summation of AMPAR- and NMDAR-mediated EPSPs during HFS has been observed previously, for example, in the CA1 region of the hippocampus (Herron *et al.* 1986) and cerebellar granule cells (D'Angelo *et al.* 1995). The summated multiple EPSC waveforms in the present studies have an initial amplitude of ~300 pA and a half-time of decay of 53.9 ± 3.5 ms, $n = 5$. In the presence of AP5 and the resulting block of NMDARs, the remaining summated AMPAR-mediated EPSCs during each HFS had a much

shorter duration than in the absence of AP5 (Fig. 1*C*ii), with the half-time of decay of the summated AMPAR-mediated EPSC waveform during the HFS being 30.5 ± 2.1 ms ($n = 5$), a value significantly smaller than in the absence of AP5 ($P < 0.05$). The much shorter duration of the summated EPSC during HFS in AP5 compared with control is clearly shown in Fig. 1*C*iii, in which the summated waveform in control and in AP5 are superimposed. Subtraction of the summated EPSC waveform in the presence of AP5 from the control summated EPSC waveform revealed the NMDAR component of the waveform, i.e. the summated NMDAR-mediated EPSC (Fig. 1*C*iv), which had a peak amplitude of ∼200 pA and a decay half-time of 46.2 ± 6.3 ms ($n = 5$), a significantly longer duration than the isolated summated AMPAR-mediated EPSC ($P < 0.05$).

Induction of LTD by a HFS–depolarisation pairing protocol evoking a low level of NMDAR activation

A pairing of HFS and postsynaptic depolarisation was an effective stimulus for the induction of NMDAR-dependent LTD providing that a low level of NMDAR activation was evoked by the pairing procedure. This was achieved by pairing the same series of HFS used to evoke LTP with a single mild step depolarisation of 1.1 s from −70 to −50 mV (see Methods). LTD was induced by this protocol with a mean amplitude of 22.1 ± 9.6 % ($n = 5$, $P < 0.05$; Fig. 2*A*). Such LTD was NMDAR dependent as it was blocked in the presence of AP5, the EPSC measuring 104.1 ± 4.1 %, $P > 0.05$, at 20 min post-pairing (Fig. 2*B*).

The summated multiple EPSC waveform during each HFS (Fig. 2*C*i) in control medium had a smaller amplitude (−60 pA) and a more rapid half-time of decay (∼35 ms) than in the previous experiments involving LTP induction. In the presence of AP5, the summated multiple AMPAR-mediated EPSC waveform during each HFS had a much shorter duration than in the absence of AP5 (Fig. 2*C*ii), with a half-time of 27.5 ± 5.1 ms, $n = 5$, $P < 0.05$. The much shorter duration of the summated EPSC during HFS in AP5 compared with control is clearly shown in Fig. 2*C*iii, in which the summated waveform in control and in AP5 are superimposed.

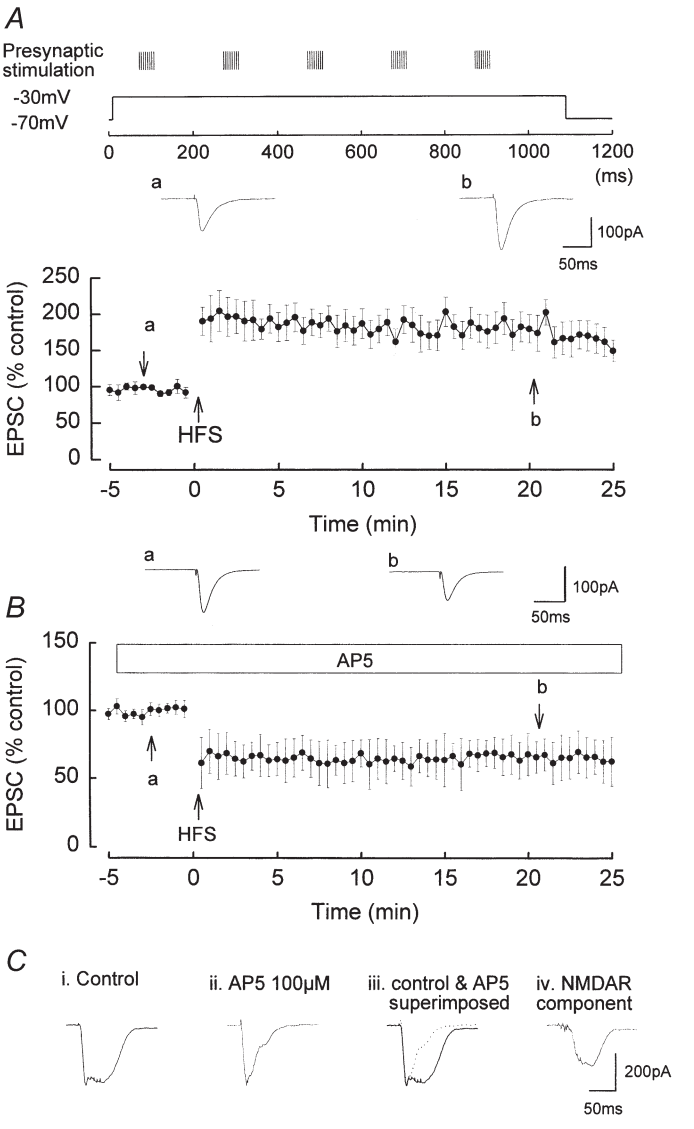

Figure 1. Induction of NMDAR-dependent LTP by a pairing protocol consisting of HFS and a single step postsynaptic depolarisation

Presynaptic stimulation was set to evoke EPSCs of ∼100 pA amplitude during test stimulation. Five trains of HFS were given, each train consisting of eight stimuli at 200 Hz and the inter-train interval of 200 ms. The presynaptic stimulation was increased to evoke ∼300 pA EPSCs during the HFS. The holding potential of the postsynaptic cell was −70 mV, and a single depolarisation to −30 mV and of 1.1 s duration was applied during the HFS. *A*, in control media, LTP was induced with a mean amplitude of 165 ± 19.1 %, $n = 7$. *a* and *b* are original traces of EPSCs prior to and following LTP induction. *B*, in the presence of the NMDAR antagonist AP5 (100 μM), LTP was completely blocked, and an NMDAR-independent LTD was evoked with a mean amplitude of 37.9 ± 15.6 %, $n = 6$. *a* and *b* are original traces of EPSCs prior to and following LTD induction. *C*, examples of summated EPSCs during a single train of HFS. *C*i shows the summated EPSCs during the initial HFS in control media, the waveform being composed of both AMPAR- and NMDAR-mediated components. *C*ii shows the summated AMPAR component of the EPSCs in the presence of AP5. Note the much shorter time course of the summated EPSCs. *C*iii shows the superimposition of the summated EPSPs in control and in AP5. *C*iv shows the NMDAR-mediated component of the summated EPSCs of the train, obtained by subtracting the summated EPSCs in AP5 from the control summated EPSCs.

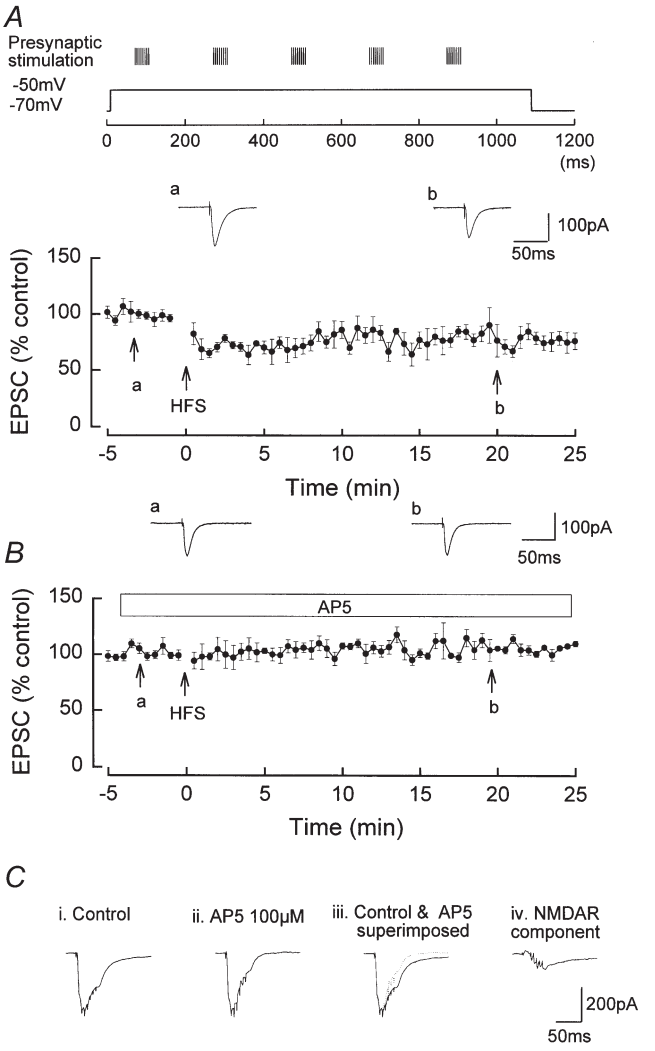

Figure 2. Induction of NMDAR-dependent LTD by the pairing of HFS and a single step depolarisation of 1.1 s duration and mild amplitude (from –70 to –50 mV)

The presynaptic stimulation was increased to evoke ~300 pA EPSCs during the HFS. *A*, LTD of mean amplitude 22.1 ± 9.6 %, $n = 5$, induced by HFS applied on the depolarising phase of a single step depolarisation of 1.1 s duration. *a* and *b* are original traces of EPSCs prior to and following LTD induction. *B*, inhibition of the LTD by AP5 (100 μM), mean amplitude 104.1 ± 4.1 %, $n = 6$. *a* and *b* are original traces of EPSCs prior to and following HFS. *C*i shows the summated EPSCs during the initial HFS in control media, composed of both AMPAR- and NMDAR-mediated components. *C*ii shows the summated AMPA receptor component of the EPSCs in the presence of AP5. *C*iii shows the superimposition of the summated EPSPs in control and in AP5. *C*iv shows the NMDAR component of the summated EPSCs of the train, obtained by subtracting the summated EPSCs in AP5 from the control summated EPSCs.

Subtraction of the summated EPSC waveform in the presence of AP5 from the control summated EPSC waveform revealed the NMDAR-component of the waveform, i.e. the summated NMDAR-mediated EPSCs (Fig. 2*C*iv), which had a very small peak amplitude (~30 pA) and a decay half-time of 39.8 ± 3.6 ms ($n = 5$). The peak amplitude and duration of this summated NMDAR-mediated EPSC was much smaller than that occurring during LTP induction.

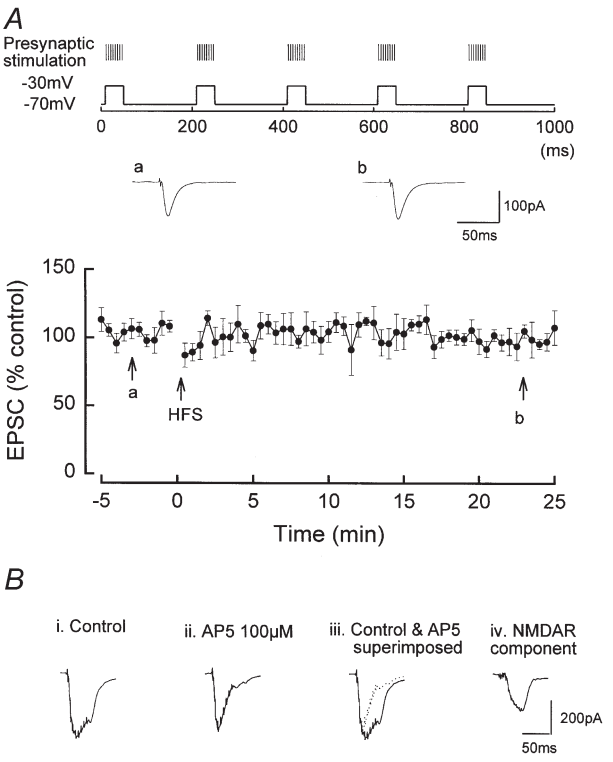

Figure 3. Induction of the LTD–LTP crossover point by the pairing of HFS and a series of depolarising trains

A, induction of the LTD–LTP crossover point by a protocol in which the presynaptic stimulation was increased to evoke ~300 pA EPSCs during the trains of HFS which were applied on the depolarising phase of a series of 40 ms duration depolarising steps from −70 to −30 mV. Mean amplitude of the EPSC at 25 min HFS was not significantly altered from the test level (107.1 ± 7.8 %, $n = 11$). *a* and *b* are original traces of EPSCs prior to and following HFS. *B*i shows the summated EPSCs during the initial HFS in control media, composed of both AMPAR- and NMDAR-mediated components. *B*ii shows the summated AMPAR component of the EPSCs in the presence of AP5. *B*iii shows the superimposition of the summated EPSPs in control and in AP5. *B*iv shows the NMDAR component of the summated EPSCs of the train, obtained by subtracting the AMPAR summated EPSCs from the combined AMPAR and NMDAR summated EPSCs.

Induction of the LTD–LTP crossover point by a HFS–depolarisation pairing protocol evoking moderate NMDAR activation

A pairing of HFS and postsynaptic depolarisation was an effective stimulus for the induction of the NMDAR-dependent LTD–LTP crossover point providing that moderate NMDAR activation was evoked by the pairing procedure. This was achieved by pairing an identical series of HFS trains used to induce LTP and LTD with a series of short step depolarisations (see Methods), designed to abbreviate activation of the NMDARs. Five strong intensity HFS trains were paired with the depolarising phase of five short (40 ms) depolarising steps each from −70 to −30 mV. This protocol resulted in an unchanged test EPSC, with no significant induction of LTP or LTD, the post-stimulus EPSC measuring 107.1 ± 7.8 % ($n = 11$, $P > 0.05$; Fig. 3*A*). However, although there was a lack of induction of synaptic plasticity, NMDARs were moderately activated during this stimulation protocol, as shown by isolation of the NMDAR current during the HFS. The summated multiple EPSC waveform, composed of both AMPAR and NMDAR components, had an initial large amplitude (~300 pA) but a relatively short duration (~40 ms) as the imposed short step depolarisations resulted in an abrupt termination of the NMDAR component of the waveform (Fig. 3*B*i). In the presence of AP5, the summated multiple AMPAR-mediated EPSCs during each HFS had a much reduced duration (Fig. 3*B*ii and iii). Subtraction of the summated EPSC waveform in the presence of AP5 from the control summated EPSC waveform revealed the NMDAR-component of the waveform, i.e. the summated NMDAR-mediated EPSCs (Fig. 3*B*iv), which had a maximum amplitude of ~200 pA and a decay half-time of 40.3 ± 9.5 ms, $n = 5$.

Lack of LTP or LTD induction by a HFS–hyperpolarisation pairing protocol which did not evoke NMDAR activation

Pairing high intensity HFS with a single hyperpolarising step of 1.1 s from −70 to −90 mV did not induce significant LTP or LTD, the post-stimulus EPSCs measuring 98.4 ± 8.7 % ($P > 0.05$, $n = 5$), at 25 min post-HFS (Fig. 4*A*). The absence of induction of LTP or LTD was associated with a lack of activation of NMDARs during the HFS. The summated multiple EPSC waveform had an initial large amplitude (~300 pA) but a relatively short duration (~40 ms; Fig. 4*B*i). In the presence of AP5, in which there was also no induction of LTP or LTD (99.1 ± 4.7 %), the summated multiple AMPA receptor-mediated EPSCs during each HFS had a very similar amplitude and duration to control (Fig. 4*B*ii and iii). Subtraction of the summated EPSC waveform in the presence of AP5 from the control summated EPSC waveform resulted in an absence of NMDAR-mediated current (Fig. 4*B*iv).

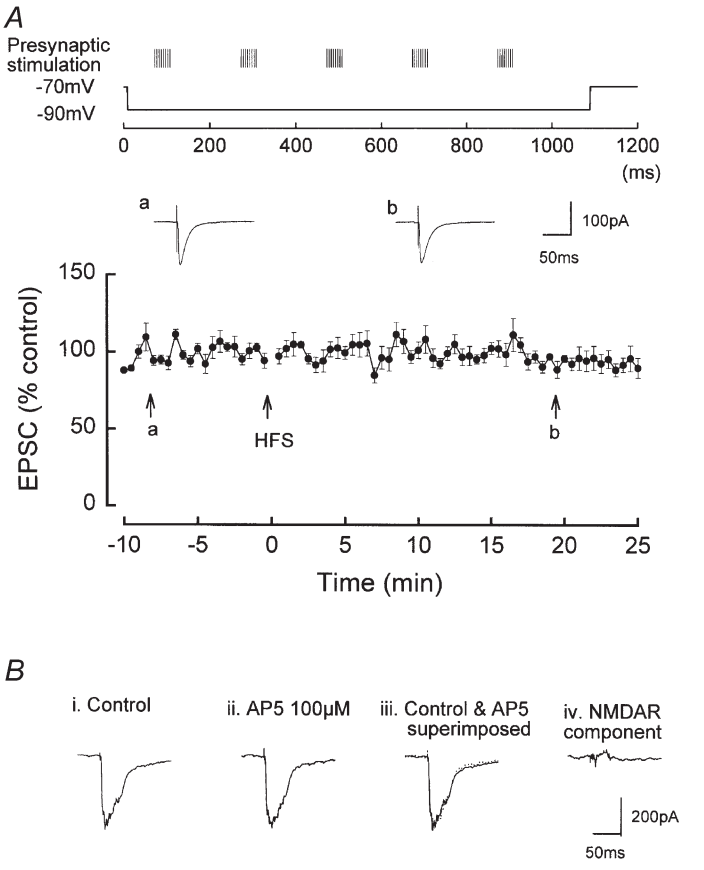

Figure 4. The absence of induction of LTP or LTD by a pairing protocol consisting of high intensity presynaptic HFS and a single step postsynaptic hyperpolarisation

The presynaptic stimulation was increased to evoke ~300 pA EPSCs during the HFS. The holding potential of the postsynaptic cell was −70 mV, and a single step hyperpolarisation to −90 mV and of 1.1 s duration was applied during the HFS. *A*, no LTP or LTD was induced by the HFS, the post-stimulus EPSCs measuring 98.4 ± 8.7 % ($P > 0.05$, $n = 5$) at 25 min post-HFS. *a* and *b* are original traces of EPSCs prior to and following HFS. *B*, examples of summated EPSCs during a single train of HFS. *B*i shows the summated EPSCs during the initial HFS in control media. *B*ii shows the summated AMPAR component of the EPSCs in the presence of AP5. *B*iii shows the superimposition of the summated EPSPs in control and in AP5. Note that the two traces are virtually identical. *B*iv shows that subtracting the waveform of the summated EPSCs in AP5 from the control summated EPSCs results in no NMDAR-mediated current.

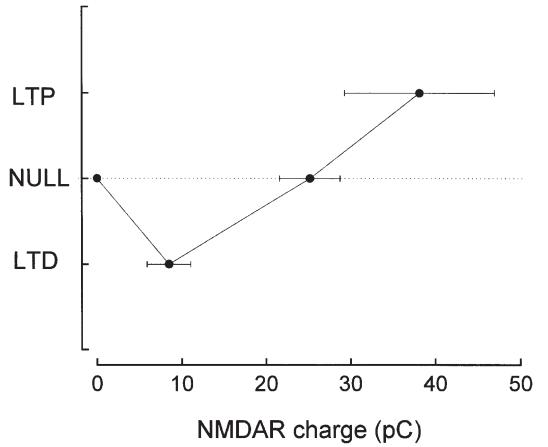

Figure 5. Synaptic plasticity–NMDAR activation curve

The amplitude of LTP, LTD or zero plasticity induced by the different stimulation protocols was plotted against the NMDAR charge transfer. Each point is the mean of $n = 5$. Note the increasing NMDAR charge transfer as plasticity alters from induction of LTD to the LTD–LTP crossover point and to LTP.

Correlation of NMDAR activation with induction of plasticity

In order to correlate quantitatively the degree of activation of the NMDAR with the induction of synaptic plasticity, the total NMDAR charge transfer was measured from the isolated NMDAR currents evoked under the different stimulation protocols which induced LTP, LTD or the LTD–LTP crossover point. This measure of NMDAR charge transfer was plotted against the amplitude and direction of plasticity, as shown in Fig. 5, in a plasticity *versus* NMDAR activation curve. It can be seen that a transition from induction of LTD to LTP via a crossover point occurs as the charge transfer via the NMDAR is increased. Particularly notable is that NMDAR-dependent LTD is associated with a very small NMDAR charge transfer, and that the LTD–LTP

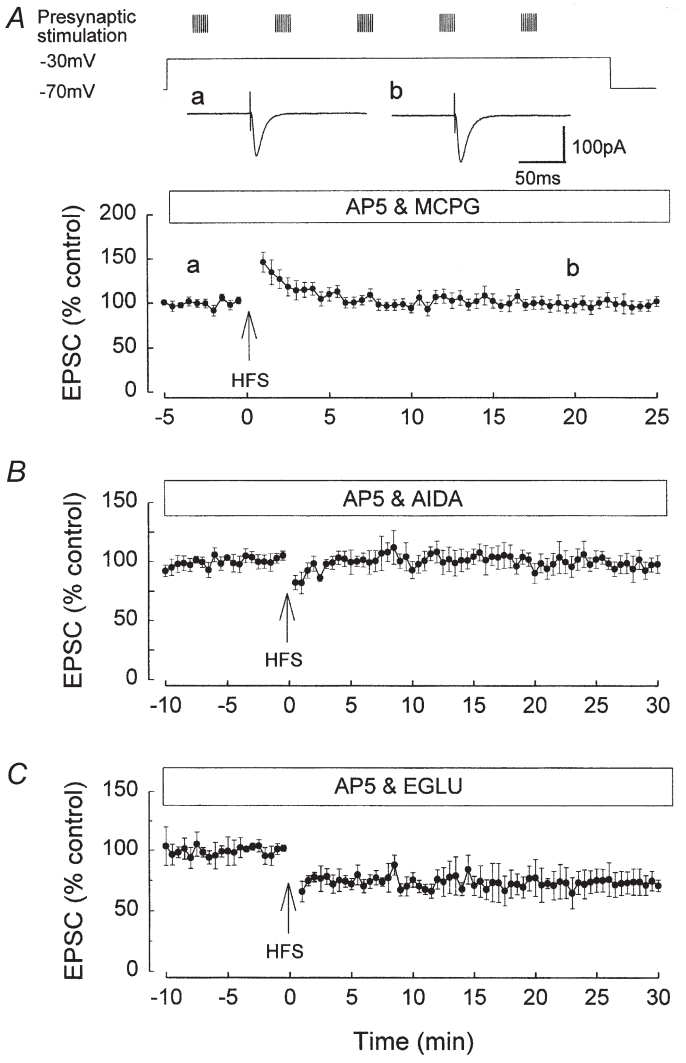

Figure 6. NMDAR-independent LTD induced by high intensity HFS paired with a single depolarisation from –70 to –30 mV is dependent upon activation of group I mGluR, but not group II

A, the induction of NMDAR-independent LTD by HFS was inhibited by the mGluR antagonist MCPG. The traces *a* and *b* show the original EPSCs prior to and following HFS, and are not significantly different. *B*, the induction of NMDAR-independent LTD was inhibited by the group I mGluR antagonist AIDA. *C*, the induction of NMDAR-independent LTD was not inhibited by the group II mGluR antagonist EGLU.

crossover point is associated with relatively large NMDAR activation, although less than that occurring during LTP induction.

High intensity HFS paired with depolarisation induces group I mGluR-dependent LTD under conditions of NMDAR inhibition

As shown in Fig. 1*B*, the pairing protocol consisting of HFS and a single step depolarisation from −70 to −30 mV induced an NMDAR-independent LTD in the presence of AP5. Such LTD was found to be dependent upon activation of mGluR, being inhibited by the mGluR antagonist MCPG (500 μM). Thus in MCPG, perfused for at least 30 min prior to HFS in the presence of AP5, the EPSC measured 102.5 ± 4.7 % at 25 min post-HFS, a value not significantly different from the pre-pairing baseline value (Fig. 6*A*).

The effects of subgroup-selective mGluR antagonists were examined on the induction of the NMDAR-independent LTD. The NMDAR-independent LTD was blocked by the selective group I mGluR antagonist AIDA (Pelliacciari *et al.* 1996). Thus in the presence of AIDA (450 μM), perfused for at least 30 min prior to pairing, the EPSC measured 101.8 ± 7.4 %, $n = 5$, at 25 min post-pairing, not significantly different from the pre-pairing baseline value (Fig. 6*B*).

The mGluR-dependent LTD was not blocked by the selective group II mGluR antagonist EGLU (Jane *et al.* 1996). In the presence of EGLU (250 μM), which was perfused for at least 30 min pre-HFS, the pairing of high intensity HFS and depolarisation induced LTD, the ESPC measuring 75.4 ± 9.5 % ($n = 5$), at 25 min post-HFS, a value not significantly different from control (Fig. 6*C*).

Induction of mGluR-dependent LTD Ca^{2+} influx via L-type Ca^{2+} channels and a rise in intracellular Ca^{2+}

The experiments shown in Fig. 4*A* demonstrated that the mGluR-dependent LTD induction required depolarisation, suggesting that Ca^{2+} influx is necessary for the LTD induction. Accordingly, the effects of the L-type voltage-gated Ca^{2+} channel inhibitor nifedipine were investigated on the induction of the LTD. The mGluR-dependent LTD was blocked by nifedipine. In the presence of nifedipine (10 μM), which was perfused for at least 30 min pre-pairing, the pairing of high intensity HFS and depolarisation failed to induce LTD, the EPSC measuring 97.8 ± 6.8 % ($n = 6$) at 25 min post-HFS (Fig. 7*A*).

The mGluR-dependent LTD was also blocked if a rise in postsynaptic intracellular Ca^{2+} was prevented. Following postsynaptic injection of the Ca^{2+} chelator BAPTA (20 mM), the HFS pairing failed to induce LTD, the EPSC measuring 98.7 ± 5.6 % ($n = 6$), at 25 min post-HFS (Fig. 7*B*).

Quantal analysis involving minimal stimulation

In order to obtain information about the site of expression of mGluR-dependent LTD, quantal analysis involving minimal stimulation techniques was carried out. Both the failure rate and potency (defined as the EPSC amplitude excluding failures (Stevens & Wang, 1994) were measured before and following mGluR-dependent LTD induction.

In all cells LTD was associated with both a decrease in potency and an increase in failure rate. Figure 8*A* shows the complete time course of one typical experiment. The mean EPSC amplitude decreased by 64 % in this cell following the induction of LTD. LTD was accompanied by an increase in the failure rate, from 14 % during the baseline period to 64 % after the pairing, and by a decrease in the potency, from 11.5 pA at baseline to 4.1 pA after the pairing. The plot of the pre-pairing period and post-pairing period data in the form of amplitude histograms confirmed the increase in failure

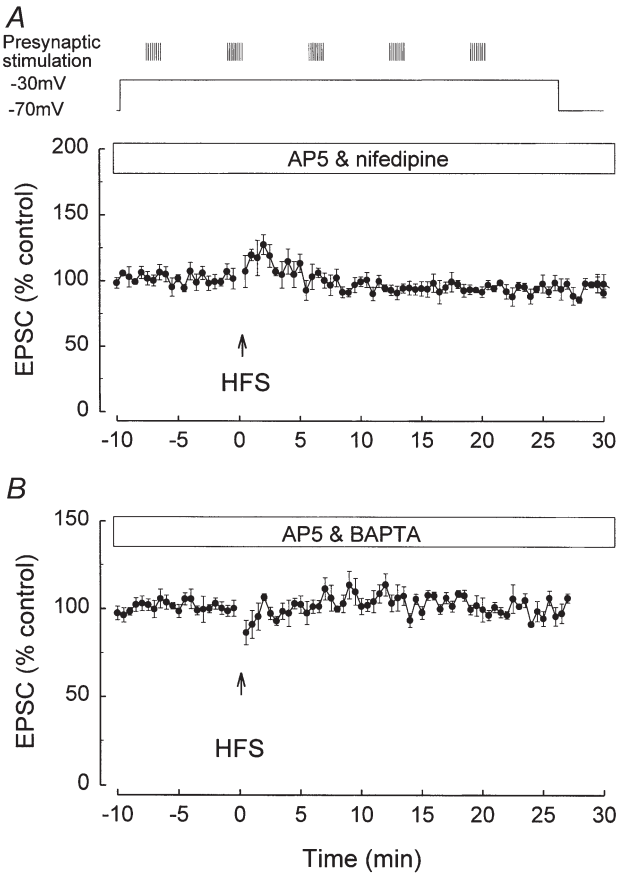

Figure 7. NMDAR-independent LTD induction is dependent upon an influx of Ca^{2+} via L-type Ca^{2+} channels and an increase in intracellular Ca^{2+}

A, the induction of NMDAR-independent LTD was inhibited by the L-type Ca^{2+} channel blocker nifedipine (10 μM). *B*, the induction of NMDAR-independent LTD was inhibited by the Ca^{2+} chelator BAPTA.

rate and the decrease in potency occurring following the induction of LTD (Fig. 8*B*). For all the five cells in which LTD was successfully induced using minimal stimulation, failure rate increased from 20.0 ± 5.4 to $52.6 \pm 8.2\%$, and the potency decreased from 24.5 ± 3.2 to 12.7 ± 2.4 pA ($P < 0.005$).

In order to ensure that the pairing procedure itself did not result in a change in potency or failure rate in the absence of LTD induction, minimal stimulation experiments were also carried out under conditions in which LTD induction was blocked by MCPG. Figure 9*A* shows the complete time course of one experiment. No significant change of the mean EPSC, the failure rate or the potency was induced by pairing in the presence of MCPG. This lack of significant change was confirmed in the plot of the amplitude histogram for pre-pairing and post-pairing periods (Fig. 9*B*). Thus in all the five cells in which stimulation was paired in the presence of MCPG, the failure rate was $25.5 \pm 5.7\%$ pre-pairing and $25.3 \pm 6.6\%$ post-pairing. The potency was 18.7 ± 1.9 pA pre-pairing and 18.9 ± 0.9 pA post-pairing.

DISCUSSION

The present studies have shown that a pairing stimulation procedure of brief HFS and depolarisation can induce NMDAR-dependent LTP, LTD, LTD–LTP crossover and also an mGluR-dependent LTD depending on the particular protocol of the induction stimulation.

NMDAR activation during the induction of plasticity

The quantitative measurement of NMDAR activity during induction of LTP, LTD and the LTP–LTD crossover point was made under conditions in which all three types of plasticity were induced by an identical level of presynaptic stimulation in order to ensure that the same number of synapses were stimulated during the induction of the three types of plasticity. Thus only the level and pattern of postsynaptic depolarisation were

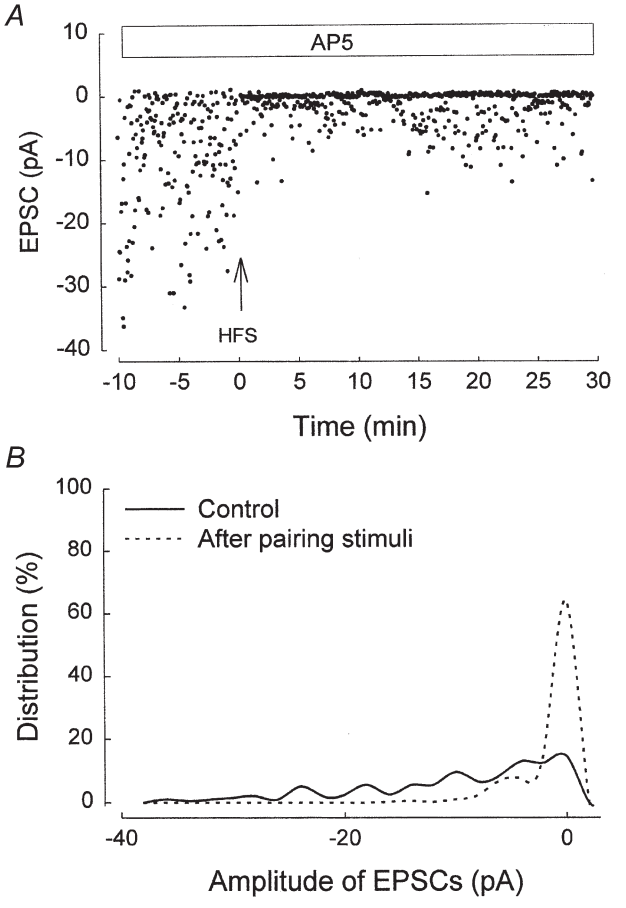

Figure 8. mGluR-dependent LTD is associated with a decrease in potency and an increase in failure rate

An example of an experiment using minimal stimulation. *A*, individual EPSC amplitude plotted against time during the whole experiment. *B*, amplitude histogram for this experiment (bin width = 1 pA).

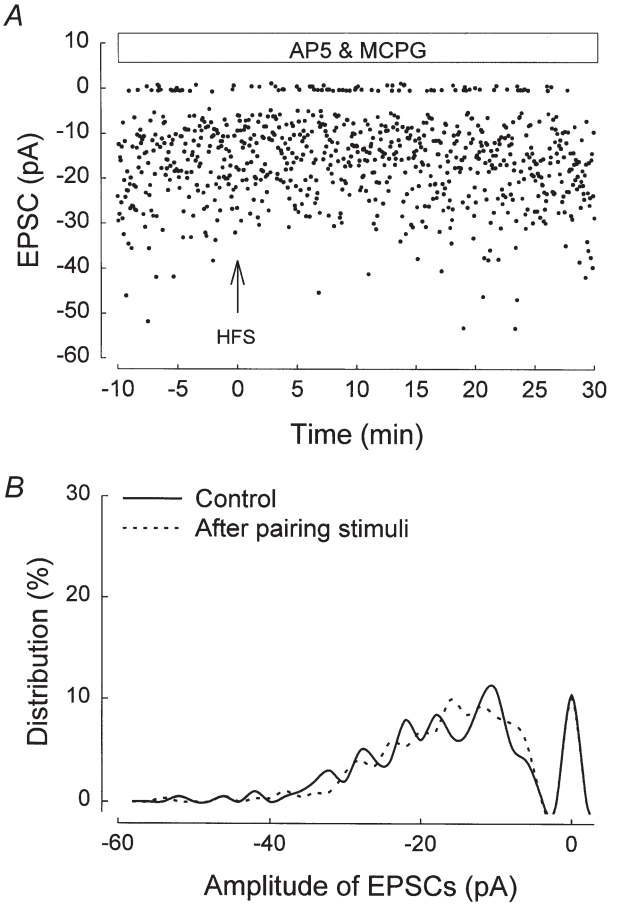

Figure 9. Inhibition of mGluR-dependent LTD by MCPG prevents the change in potency and failure rate following HFS

An example of an experiment using minimal stimulation. *A*, individual EPSC amplitude plotted against time during the whole experiment in the presence of MCPG. *B*, amplitude histogram for this experiment (bin width = 1 pA).

modified to induce either LTP, LTD or the LTD–LTP crossover point.

NMDAR charge transfer was found to increase as the NMDAR-dependent plasticity underwent a transition from no plasticity to LTD, and then from LTD to LTP via an LTD–LTP crossover point, as shown in Fig. 5. Particularly notable is the very low level of NMDAR activation associated with the induction of LTD compared with that during the induction of LTP, the NMDAR charge transfer ratio for LTD compared to LTP being 0.22. Also notable is that the LTD–LTP crossover point was associated with a relatively high level of activation of NMDARs compared to that for the induction of LTD. No LTD or LTP was induced if HFS was applied at a hyperpolarised membrane potential, in agreement with several previous studies (Malinow & Miller, 1986; Artola *et al.* 1990; Mulkey & Malenka, 1992; Ngezahayo *et al.* 2000), demonstrating that LTD/LTP induction requires a minimum level of membrane depolarisation. The inability of HFS given at hyperpolarised potentials to induce LTP/LTD was shown in the present studies to be associated with a complete absence of NMDAR activation.

The curve relating plasticity to NMDAR activation shown in Fig. 5 shows a strong resemblance to the modified Bienenstock-Cooper-Munro (BCM) function (Bear, 1995), in which a smooth transition from LTD to LTP induction occurs as the frequency of stimulation is increased. The present experiments are strong quantitative support for the theory of Bear (1995) that the transition from LTD to LTP with increased frequency is due to an increased NMDAR activation, and that the LTP–LTD crossover frequency is associated with a critical level of NMDAR activation (reviewed by Bear & Malenka, 1994; Bear, 1995).

It is well established that Ca^{2+} influx via NMDARs is required to trigger the induction of NMDAR-dependent LTP or LTD (Lynch *et al.* 1983; Malenka *et al.* 1983; Artola & Singer, 1993). According to the differential threshold hypothesis, LTP has a high intrinsic Ca^{2+} threshold and is induced by a large rise in Ca^{2+}, whereas LTD has a low intrinsic Ca^{2+} threshold and is selectively induced by a lower rise in Ca^{2+} (Lisman, 1989; Artola & Singer, 1993). Measurements of Ca^{2+} increase induced by stimulation protocols which induce either LTP or LTD have supported this theory, although recent studies have shown that the rate of rise and/or the duration of Ca^{2+} increase is also important in determining the polarity of synaptic plasticity (Hansel *et al.* 1997; Otani & Connor, 1998; Yang *et al.* 1999). Peak dendritic Ca^{2+} levels have been shown to rise by 37 % and 62 % (ratio 0.6) following LTD and LTP induction, respectively, in the visual cortex (Hansel *et al.* 1997), and to 464 nM and 1.25 μM (ratio 0.37) during LTD and LTP, respectively, in the CA1 region of the hippocampus (Otani & Connor, 1998). The ratio of Ca^{2+} increase associated with LTD/LTP induction is somewhat larger in these studies than the ratio of NMDAR activation associated with LTD/LTP induction measured in the present experiments (0.22). This suggests that the increase in Ca^{2+} levels occurring during LTD induction may be due in part to Ca^{2+}-induced Ca^{2+} release from intracellular Ca^{2+} stores, with Ca^{2+} influx the via NMDAR serving as the trigger for Ca^{2+} release. The necessity of Ca^{2+}-induced Ca^{2+} release from intracellular ryanodine-sensitive Ca^{2+} stores for the induction of LTD has been previously demonstrated in the dentate gyrus using pharmacological inhibitors of the ryanodine receptor (O'Mara *et al.* 1995; Wang *et al.* 1997).

Induction of LTD by HFS

Most previous studies have shown a clear division of the stimulation parameters resulting in LTP and LTD in the CA1 or dentate gyrus of the hippocampus, with NMDAR-dependent LTP induced by brief HFS of 50–200 Hz or the pairing of brief LFS and large postsynaptic depolarisation (Bliss & Lomo, 1973; Wigstrom *et al.* 1985; Kelso *et al.* 1986; reviewed by Bliss & Collingridge, 1993), and NMDAR-dependent LTD induced by several minutes of LFS at 1–5 Hz (Dudek & Bear, 1992*a*,*b*; Mulkey & Malenka, 1992; O'Mara *et al.* 1995; Huang *et al.* 1997; reviewed by Linden & Connor, 1995; Bear & Abraham, 1996; Anwyl, 1999). In the present studies, either LTP or LTD could be induced by brief HFS protocols without media or pharmacological changes simply by modifying the duration or amplitude of postsynaptic depolarisation. This finding supports other recent studies in which LTD/depotentiation could be induced by HFS *in vitro*, although only under altered media or pharmacological conditions. Thus HFS applied in the presence of a low concentration of AP5 (Cummings *et al.* 1996), or HFS applied on the trough of a cholinergically evoked theta rhythm (Huerta & Lisman, 1995), resulted in the induction of LTD and depotentiation, respectively. In addition, HFS applied *in vivo* was found to induce depotentiation when applied on the trough of the tail pinch-evoked theta rhythm (Holscher *et al.* 1997). Such findings raise the possibility that HFS may be a strong physiological stimulus for the induction of LTD as well as LTP *in vivo*, with the level of presynaptic activation and the pattern and amplitude of postsynaptic depolarisation determining the direction of plasticity.

The present studies showing that an mGluR-dependent form of LTD induction can be induced in the dentate gyrus in addition to an NMDAR-dependent LTD induction adds strongly to the growing evidence for an mGluR-dependent form of LTD which is distinct from an NMDAR-dependent LTD (Bolshakov & Siegelbaum, 1994; O'Mara *et al.* 1995; Oliet & Nicoll, 1997; Malenka, 1998; Camodeca *et al.* 1999). In the present studies, the mGluR-dependent LTD induction was only induced by a pairing procedure involving high intensity presynaptic stimulation. Such stimulation may result in spillover of extracellular glutamate to the perisynaptic location of the

group I mGluRs (Lujan *et al.* 1996) on adjacent axon terminals. The demonstration of an involvement of group I mGluRs in the LTD induced by HFS is in agreement with previous studies showing that LFS-induced LTD can be induced via the activation of group I mGluRs in the medial perforant path of the dentate gyrus (Camodeca *et al.* 1999) and CA1 (Oliet *et al.* 1997). The lack of involvement of group II mGluRs in HFS-induced LTD in the present studies is surprising in view of the high density of such receptors at the medial perforant path synapses (Shigemoto *et al.* 1997) and that prolonged LFS-induced LTD was found to involve group II mGluRs at this synapse (Huang *et al.* 1997, 1999). It is possible that the HFS induction protocol for LTD used in the present studies is too brief for the sufficient activation of the group II mGluRs.

The influx of Ca^{2+} required for the mGluR-dependent LTD was shown to be via L-type Ca^{2+} channels in the present study, in agreement with one previous study involving LFS-induced mGluR-dependent LTD in CA1 (Bolshakov & Siegelbaum, 1994). However, under certain conditions of stimulation, Ca^{2+} entry via T-type Ca^{2+} channels can support mGluR-dependent LTD. Thus in previous studies in the dentate gyrus (Wang *et al.* 1997, 1998) and CA1 (Oliet *et al.* 1997), mGluR-dependent LFS-induced LTD evoked under conditions of mild postsynaptic depolarisation was blocked by the T-type Ca^{2+} channel inhibitor Ni^{2+}. It therefore appears that Ca^{2+} influx via L- or T-type Ca^{2+} channels can participate in mGluR-dependent LTD induction, with T-type Ca^{2+} channels involved when postsynaptic depolarisation is mild and L-type Ca^{2+} channels involved when depolarisation is strong.

The expression of the mGluR-dependent LTD was associated with both a decrease in potency and an increase in failure rate. The decrease in potency could be caused by a decrease in q (quantal size) or n (number of functional synapses) providing that a single synapse was being activated during the minimal stimulation, although if more than one synapse was being activated, then the decrease in potency could also be caused by a decrease in the probability of release. Although the increase in failure rate could classically be due to a decrease in the probability of release, under the silent synapse theory of Liao *et al.* (1995), it could also be caused by a postsynaptic change associated with a failure of detection of released transmitter as active synapses are converted into silent synapses. In previous studies in the hippocampus, a similar decrease in potency and increase in failure rate accompanying NMDAR-dependent LTD induction was detected (Man *et al.* 2000) and attributed to a postsynaptic decrease in the number of active synapses. Moreover, immunocytochemical studies have provided strong evidence that LTD is associated with a complete removal of AMPAR clusters (Carroll *et al.* 1999).

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